

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF

Hiroshi Izui, et al

:GROUP ART UNIT: 1636

SERIAL NO: 09/641,892

:EXAMINER: Lambertson, David A

FOR: METHOD FOR PRODUCING L-GLUTAMIC ACID BY FERMENTATION

ACCOMPANIED BY PRECIPITATION

DECLARATION UNDER 37 C.F.R. SECTION 1.132

ASSISTANT COMMISSIONER FOR PATENTS

WASHINGTON, D.C. 20231

SIR

I, Yoshihiko Hara of c/o Ajinomoto Co., Inc., 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Kanagawa, who deposes and states that:

1. I am a graduate of Tokyo University, Japan, where I majored in microbiology and molecular biology.

2. I have been employed by Ajinomoto Co., Inc. for 6 years as a researcher in the fields of microbiology and molecular biology.

3. I have understood the Office Action dated March 19, 2004.

4. The following experiments were conducted by me or under my direct supervision and control.

Screening for the strains with improved resistance to  
L-glutamic acid at a high concentration in a low pH environment

In the present experiment, mutants derivative from Enterobacter agglomerans SC17sucA/RSFCPG+pSTVCB (named G1 to G35, G106, and S1 to S44) and Enterobacter agglomerans SC17sucA/RSFCPG+pSTVCB (named P) strains are used. G106 strain is disclosed as AJ13601 (FERM BP-7207) in the Examples of the present invention.

These strains were cultured at 34 °C during certain time at 5ml volume in 3 kinds of mediums: glucose medium (pH4.7, glucose 3.77 g/L), sucrose medium (pH4.7, sucrose 4.21 g/L) and MS medium (neutral pH, sucrose 41.4 g/L) as the procedures in Example <4> of the present specification. Then, we determined O.D., remained glucose (RG), remained sucrose (RS), and accumulated glutamic acid (Glu) in the medium. The results are shown in the attached sheets. It should be noted that the values O.D. were measured without dilution, except for those of MS medium.

5. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of application or any patent issuing thereon.

Date: September 8, 2004

Yoshihiko Hara

Yoshihiko Hara

## The attached sheets

No	Strain	Med-G (17hr)		Med-S (26hr)	Med-S (40hr)		Med-MS			
		OD	RG (g/L)	OD	OD	RS (g/L)	OD (x51)	Glu (g/L)	RS (g/L)	Yield (%)
1	G1	0.18	3.08	0.06	0.05	3.90	0.249	23.3	0.0	56.3
2	G2	0.16	3.18	0.06	0.06	3.87	0.255	22.7	0.0	54.8
3	G3	0.29	2.75	0.07	0.06	3.91	0.243	17.6	0.1	42.6
4	G4	0.23	2.85	0.06	0.06	3.93	0.266	22.5	0.0	54.3
5	G5	0.25	2.88	0.05	0.05	3.92	0.265	22.1	0.0	53.3
6	G6	0.17	3.17	0.04	0.04	3.93	0.269	22.3	0.0	53.8
7	G7	0.15	3.37	0.06	0.06	3.99	0.283	22.3	0.0	53.8
8	G8	0.28	0.93	0.04	0.04	3.91	0.294	21.8	0.0	52.8
9	G9	0.15	3.31	0.06	0.06	3.95	0.270	22.5	0.0	54.3
10	G10	0.26	3.08	0.06	0.07	3.94	0.259	22.1	0.0	53.3
11	G11	0.43	2.02	0.06	0.06	3.88	0.249	17.4	0.0	42.1
12	G12	0.26	2.74	0.09	0.09	3.92	0.250	22.5	0.0	54.3
13	G13	0.33	2.65	0.06	0.06	3.95	0.250	17.0	0.1	41.1
14	G14	0.50	1.86	0.06	0.05	3.96	0.244	17.0	0.1	41.1
15	G15	0.21	3.11	0.08	0.09	3.94	0.257	22.7	0.0	54.8
16	G16	0.44	2.03	0.07	0.07	3.89	0.248	17.0	0.1	41.1
17	G17	0.18	3.40	0.05	0.05	3.91	0.300	21.2	0.0	51.3
18	G18	0.23	2.92	0.06	0.06	3.94	0.284	22.3	0.0	53.8
19	G19	0.18	3.12	0.07	0.08	3.96	0.276	22.3	0.1	53.8
20	G20	0.41	2.22	0.06	0.06	3.87	0.248	17.6	0.1	42.6
21	G21	0.66	0.46	0.08	0.08	3.91	0.273	20.0	0.0	48.2
22	G22	0.17	2.95	0.05	0.05	3.93	0.256	21.6	0.0	52.3
23	G23	0.14	3.39	0.06	0.07	3.93	0.257	22.3	0.0	53.8
24	G24	0.38	2.62	0.08	0.08	3.98	0.250	16.6	0.0	40.1
25	G25	0.42	2.22	0.06	0.06	3.92	0.248	17.0	0.1	41.1
26	G26	0.22	2.96	0.06	0.07	3.98	0.271	22.3	0.0	53.8
27	G27	0.16	3.03	0.08	0.08	3.93	0.296	22.5	0.0	54.3
28	G28	0.31	2.79	0.06	0.06	3.94	0.268	17.0	0.1	41.1
29	G29	0.68	0.20	0.08	0.09	3.82	0.271	19.7	0.1	47.7
30	G30	0.18	3.18	0.06	0.06	3.94	0.256	22.1	0.0	53.3
31	G31	0.31	2.64	0.07	0.06	3.94	0.245	16.6	0.1	40.1
32	G32	0.34	2.66	0.07	0.07	3.97	0.256	16.2	0.1	39.1
33	G33	0.32	2.85	0.06	0.07	3.97	0.248	16.8	0.1	40.6
34	G34	0.48	0.23	0.08	0.09	3.61	0.265	18.1	0.0	43.7
35	G35	0.50	0.00	0.06	0.06	3.94	0.312	20.0	0.0	48.2
36	G106	0.52	0.00	0.07	0.07	3.93	0.281	21.4	0.0	51.8
37	P	0.12	3.42	0.04	0.04	3.98	0.282	21.0	0.0	50.8
38	P	0.12	3.41	0.04	0.05	4.02	0.264	22.1	0.0	53.3
39	P	0.13	3.33	0.05	0.05	3.98	0.263	20.6	0.0	49.7
40	N	0.01	3.77	0.00	0.00	4.21	0.000	0.0	41.4	
41	S1	0.20	3.22	0.06	0.06	4.00	0.250	21.8	0.0	52.8
42	S2	0.36	2.58	0.07	0.07	4.02	0.243	17.4	0.1	42.1
43	S3	0.15	3.27	0.05	0.05	4.00	0.251	22.3	0.0	53.8
44	S4	0.19	3.06	0.05	0.05	3.98	0.260	22.1	0.0	53.3
45	S5	0.16	3.33	0.05	0.05	4.00	0.256	22.7	0.0	54.8
46	S6	0.66	1.81	0.06	0.06	3.95	0.252	17.6	0.1	42.6
47	S7	0.15	3.26	0.06	0.05	3.99	0.266	21.2	0.0	51.3

Sheet1

48	S8	0.19	3.28	0.06	0.06	3.99	0.261	22.5	0.0	54.3
49	S9	0.18	3.23	0.06	0.06	3.96	0.249	22.3	0.0	53.8
50	S10	0.50	1.58	0.09	0.09	4.02	0.260	16.6	0.1	40.1
51	S11	0.14	3.25	0.05	0.05	4.01	0.254	22.3	0.0	53.8
52	S12	0.19	3.18	0.07	0.07	3.99	0.266	21.6	0.0	52.3
53	S13	0.25	2.92	0.06	0.06	4.04	0.251	22.7	0.0	54.8
54	S14	0.22	3.13	0.07	0.08	4.01	0.272	22.1	0.0	53.3
55	S15	0.16	3.22	0.05	0.05	4.01	0.260	22.3	0.0	53.8
56	S16	0.15	3.18	0.11	0.12	4.02	0.258	22.3	0.0	53.8
57	S17	0.21	3.11	0.06	0.06	4.03	0.261	22.3	0.0	53.8
58	S18	0.22	2.91	0.08	0.09	4.07	0.258	22.3	0.0	53.8
59	S19	0.15	3.36	0.05	0.06	3.98	0.253	22.1	0.0	53.3
60	S20	0.11	3.50	0.09	0.10	4.00	0.260	22.1	0.0	53.3
61	S21	0.21	3.39	0.07	0.07	4.01	0.261	22.1	0.0	53.3
62	S22	0.14	3.28	0.07	0.07	4.02	0.255	22.1	0.0	53.3
63	S23	0.12	3.32	0.06	0.06	4.07	0.262	22.1	0.0	53.3
64	S24	0.22	3.26	0.07	0.07	4.04	0.258	21.8	0.0	52.8
65	S25	0.25	2.93	0.09	0.09	4.01	0.254	17.4	0.1	42.1
66	S26	0.13	3.30	0.06	0.07	4.01	0.254	22.3	0.1	53.8
67	S27	0.11	3.45	0.07	0.07	4.01	0.257	22.3	0.0	53.8
68	S28	0.12	3.39	0.10	0.11	4.03	0.261	22.7	0.0	54.8
69	S29	0.19	3.06	0.06	0.07	4.01	0.250	22.5	0.0	54.3
70	S30	0.42	2.42	0.08	0.08	4.00	0.246	16.6	0.1	40.1
71	S31	0.17	3.16	0.05	0.06	4.01	0.261	21.8	0.0	52.8
72	S32	0.14	3.28	0.05	0.05	4.02	0.257	22.3	0.0	53.8
73	S33	0.48	1.62	0.08	0.08	3.83	0.253	17.9	0.0	43.1
74	S34	0.18	3.05	0.07	0.08	4.00	0.279	22.1	0.0	53.3
75	S35	0.13	3.27	0.08	0.09	4.00	0.258	21.8	0.0	52.8
76	S36	0.16	3.17	0.07	0.08	3.98	0.255	22.1	0.0	53.3
77	S37	0.28	2.79	0.07	0.07	4.01	0.262	17.0	0.1	41.1
78	S38	0.20	3.14	0.10	0.11	4.00	0.261	21.8	0.0	52.8
79	S39	0.30	2.97	0.06	0.07	3.94	0.279	22.5	0.0	54.3
80	S40	0.13	3.32	0.07	0.07	4.02	0.279	21.6	0.1	52.3
81	S41	0.20	3.08	0.06	0.06	3.96	0.264	22.1	0.0	53.3
82	S42	0.22	3.02	0.08	0.08	3.98	0.275	14.1	0.1	34.0
83	S43	0.36	2.37	0.07	0.08	3.94	0.264	16.6	0.1	40.1
84	S44	0.18	3.15	0.05	0.06	3.86	0.277	22.3	0.0	53.8

## Appendix

### 1. Experiment

*Enterobacter agglomeranse* strain SC17 was transformed with RSFCPG and pSTVCB. The transformed strain SC17/RSFCPG+pSTVCB was cultured as follows:

The strain SC17/RSFCPG+pSTVCB was inoculated into a 1-L jar fermenter containing 300 ml of medium containing 40 g/L of glucose, 5 g/L of  $(\text{NH}_4)_2\text{SO}_4$ , 1.5 g/L of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 6 g/L of  $\text{KH}_2\text{PO}_4$ , 1.5 g/L of NaCl, 0.75 g/L of  $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$ , 0.06 g/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.06 g/L of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 2.16 mg/L of  $\text{ZnSO}_4 \cdot 2\text{H}_2\text{O}$ , 1.92 mg/L of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 2.16 mg/L of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 1.2 mg/L of boric acid, 3.6 mg/L of  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 6 g/L of yeast extract, 600 mg/L of L-lysine hydrochloride, 600 mg/L of L-methionine, 600 mg/L of DL- $\alpha,\epsilon$ -diaminopimelic acid, 25 mg/L of tetracycline hydrochloride and 25 mg/L of chloramphenicol and cultured at 37°C and pH 4.7 to perform culture for L-glutamic acid production. The culture pH was controlled by introducing ammonia gas into the medium. As the initially added glucose was depleted, 600 g/L of glucose was continuously added.

### 2. Results

OD at 562 nm and L-glutamic acid accumulation of the culture were measured. The results are shown below. L-Glutamic acid precipitated as crystals was not observed.

